reduced protein production. When culturing is performed on an industrial scale, this reduced production can result in serious financial losses.

[0006] Therefore, a need exists for a simple, inexpensive solution to reduce poloxamer variability and to improve poloxamer performance, particularly for poorly performing lots.

[0007] All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

SUMMARY

[0008] The disclosure provided herein discloses, *inter alia*, methods of preparing a poloxamer (e.g., for use in a cell culture medium). Also provided are poloxamers prepared by the methods described herein. Further disclosed herein are cell culture media compositions containing a poloxamer prepared by the methods described herein. Further disclosed herein are methods of producing a polypeptide in a cell culture by culturing a cell that produces the polypeptide in a cell culture medium containing a poloxamer prepared by the methods described herein.

[0009] Accordingly, in one aspect, provided herein are methods of preparing a poloxamer for use in a cell culture medium, comprising the steps of: (a) heating a solid poloxamer to at least 60°C to form a liquid poloxamer, wherein the poloxamer is heated to:

(1) between about 157°C and about 185°C for at least 1 minute,
(2) between about 134°C and about 157°C for at least 1 minute,
(3) between about 120°C and about 134°C for at least about 62 minutes,
(4) between about 100°C and about 120°C for at least about 98 minutes,
(5) between about 80°C and about 100°C for at least about 122 minutes, or
(6) between about 60°C and about 80°C for at least about 143 minutes; and

(b) cooling the liquid poloxamer to a temperature below 50°C to form a solid heat-treated poloxamer, wherein the cooling is not conducted in a prilling or milling device, and wherein the poloxamer comprises a copolymer of ethylene oxide and propylene oxide. In some embodiments, cell viability in a cell culture medium comprising the heat-treated poloxamer is increased as compared to cell viability in a cell culture medium comprising the poloxamer before step (a). In some embodiments, the poloxamer in step (a) is heated to between about 60°C and about 185°C. In some embodiments, the poloxamer in step (a) is heated to between about 157°C and about 185°C. In some embodiments, the poloxamer is heated to between about 157°C and about 185°C for at least 1 minute. In some embodiments, the poloxamer is heated to between about 157°C and about 185°C for between 1 minute and about 250 minutes. In some embodiments, the poloxamer in step (a) is heated to between about 134°C and about 157°C. In some embodiments, the poloxamer is heated to between about 134°C and about 157°C for at least 1 minute. In some

about 25 to about 60. In some embodiments, the poloxamer has a melting temperature of about 55°C. In some embodiments, the poloxamer has an average molecular weight of from about 6,000 to about 18,000 Daltons. In some embodiments, the poloxamer comprises a copolymer having a formula of $HO(C_2H_4O)_n(C_3H_6O)_m(C_2H_4O)_nH$ with n having a value of about 80, with m having a value of about 27, and the poloxamer has an average molecular weight of from about 7680 to about 9510 g/mol. In some embodiments, the poloxamer is poloxamer 188. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a Chinese Hamster Ovary (CHO) cell. In some embodiments, the cell is an insect cell. In some embodiments, the cell produces a polypeptide.

[0010] Provided herein is a poloxamer prepared by any of the methods described above and herein.

[0011] In a further aspect, provided herein is a cell culture medium comprising a poloxamer prepared by any of the methods described above and herein. In some embodiments, the cell medium comprises the heat-treated poloxamer at about 0.1 g/L to about 10 g/L. In some embodiments, the cell medium comprises the heat-treated poloxamer at about 0.1 g/L to about 3 g/L. In some embodiments, the cell medium comprises the heat-treated poloxamer at about 3 g/L to about 10 g/L.

[0012] In a still further aspect, provided herein are methods of producing a polypeptide in a cell culture, comprising the step of culturing a cell that produces the polypeptide in a cell culture medium under conditions suitable for production of the polypeptide, wherein the cell culture medium comprises a poloxamer prepared by any of the methods described above and herein. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a Chinese Hamster Ovary (CHO) cell. In some embodiments, the cell is an insect cell. In some embodiments, the cell medium comprises the heat-treated poloxamer at about 0.1 g/L to about 10 g/L. In some embodiments, the cell medium comprises the heat-treated poloxamer at about 0.1 g/L to about 3 g/L. In some embodiments, the cell medium comprises the heat-treated poloxamer at about 3 g/L to about 10 g/L. In some embodiments, the polypeptide is an antibody or antigen-binding fragment thereof.

[0013] Accordingly, provided herein are methods of preparing a poloxamer (e.g., for use in a cell culture medium), comprising the steps of (a) heating a purified poloxamer to about 80°C or above (e.g., about 80°C to about 100°C) to form a liquid poloxamer, and (b) cooling the liquid poloxamer to a temperature at about 50°C or below to form a solid heat-treated poloxamer, wherein the cooling is not conducted in a prilling or milling device. In some embodiments, the poloxamer comprises a copolymer of ethylene oxide and propylene oxide.

[0013a] Provided herein are methods of preparing a poloxamer for use in a cell culture medium, comprising the steps of: (a) heating a poloxamer to about 80°C to about 100°C to form a liquid poloxamer; and (b) cooling the liquid poloxamer to a temperature below about 50°C to form a solid heat-treated poloxamer, wherein the cooling is not conducted in a prilling or milling device; wherein the poloxamer comprises a copolymer of ethylene oxide and propylene oxide.

[0013b] In some embodiments, the poloxamer comprises a copolymer of ethylene oxide and propylene oxide having a formula of $HO(C_2H_4O)_n(C_3H_6O)_m(C_2H_4O)_nH$, wherein n is from about 60 to about 150 and m is from about 25 to about 60. In some embodiments, cell viability in a cell culture medium comprising the heat-treated poloxamer is increased as compared to cell viability in a cell culture medium comprising the poloxamer before step (a). In some embodiments, the poloxamer is a purified poloxamer. In some embodiments, a purified poloxamer is a poloxamer composition that does not contain another therapeutic or pharmaceutical compound. In some embodiments, the poloxamer in step (a) does not contain another therapeutic or pharmaceutical compound. In some embodiments, the poloxamer in step (a) is heated to about 85°C to about 91°C. In some embodiments herein, the poloxamer is heated from about 10 to about 15 minutes. In some embodiments herein, the liquid poloxamer in step (b) is cooled at ambient temperature, about 2°C to about 8°C, or below 0°C. In some embodiments herein, the liquid poloxamer is cooled for at least about 20 minutes. In some embodiments herein, the heated-treated poloxamer produced in step (b) is added into a cell culture medium. In some embodiments herein, steps (a) and (b) are repeated at least once before adding the heated-treated poloxamer into the cell culture medium. In some embodiments herein, the cell viability is increased by at least about 10%. In some embodiments herein, the cell viability is increased by at least about 30%. In some embodiments herein, the cell viability in a cell culture medium comprising the poloxamer before step (a) is below about 80%. In some embodiments herein, the poloxamer has been treated by a prilling process before step (a). In some embodiments herein, the poloxamer has a melting temperature in the range of about 45°C to about 60°C. In some embodiments herein, the poloxamer has an average molecular weight of from about 6,000 to about 18,000 Daltons. In some embodiments herein, the poloxamer is poloxamer 188. In some embodiments, the poloxamer comprises a copolymer having a formula of $HO(C_2H_4O)_n(C_3H_6O)_m(C_2H_4O)_nH$ with n having a value of about 80, with m having a value

CLAIMS

What is claimed is:

1. A method of preparing a poloxamer for use in a cell culture medium, comprising the steps of:

(a) heating a solid poloxamer to at least 60°C to form a liquid poloxamer, wherein the poloxamer is heated to:

(1) between about 157°C and about 185°C for at least 1 minute,

(2) between about 134°C and about 157°C for at least 1 minute,

(3) between about 120°C and about 134°C for at least about 62 minutes,

(4) between about 100°C and about 120°C for at least about 98 minutes,

(5) between about 80°C and about 100°C for at least about 122 minutes, or

(6) between about 60°C and about 80°C for at least about 143 minutes; and

(b) cooling the liquid poloxamer to a temperature below 50°C to form a solid heat-treated poloxamer, wherein the cooling is not conducted in a prilling or milling device, and wherein the poloxamer comprises a copolymer of ethylene oxide and propylene oxide.

2. The method of claim 1, wherein cell viability in a cell culture medium comprising the heat-treated poloxamer is increased as compared to cell viability in a cell culture medium comprising the poloxamer before step (a).

3. The method of claim 1, wherein the poloxamer is heated to between about 157°C and about 185°C for between 1 minute and about 250 minutes.

4. The method of claim 1, wherein the poloxamer is heated to between about 134°C and about 157°C for between 1 minute and about 250 minutes.

5. The method of claim 1, wherein the poloxamer is heated to between about 120°C and about 134°C for between about 62 minutes and about 250 minutes.

6. The method of claim 1, wherein the poloxamer is heated to between about 100°C and about 120°C for between about 98 minutes and about 250 minutes.

7. The method of claim 1, wherein the poloxamer is heated to between about 80°C and about 100°C for between about 122 minutes and about 250 minutes.

8. The method of claim 1, wherein the poloxamer is heated to between about 60°C and about 80°C for between about 143 minutes and about 250 minutes.

9. The method of claim 1 or claim 2, wherein cell viability in a cell culture medium comprising the heat-treated poloxamer is increased by at least 10% as compared to cell viability in a cell culture medium comprising the poloxamer before step (a).

10. The method of claim 9, wherein the cell viability is increased by at least about 20%.

11. The method of claim 9, wherein the cell viability is increased by at least about 30%.

12. The method of any one of claims 1-11, wherein the liquid poloxamer in step (b) is cooled at ambient temperature, about 2°C to about 8°C, or below 0°C.

13. The method of any one of claims 1-12, wherein the poloxamer is heated under a vacuum.

14. The method of any one of claims 1-13, wherein the liquid poloxamer is cooled for at least about 20 minutes.

15. The method of any one of claims 1-14, wherein the heated-treated poloxamer produced in step (b) is added into a cell culture medium.

16. The method of any one of claims 1-15, wherein steps (a) and (b) are repeated at least once before adding the heated-treated poloxamer into the cell culture medium.

17. The method of any one of claims 1-16, wherein the poloxamer has been treated by a prilling process before step (a).

18. The method of any one of claims 1-17, wherein the poloxamer has a formula of $HO(C_2H_4O)_n(C_3H_6O)_m(C_2H_4O)_nH$, wherein n is from about 60 to about 150 and m is from about 25 to about 60.

19. The method of any one of claims 1-18, wherein the poloxamer has a melting temperature of about 55°C.

20. The method of any one of claims 1-19, wherein the poloxamer has an average molecular weight of from about 6,000 to about 18,000 Daltons.

21. The method of any one of claims 1-20, wherein the poloxamer comprises a copolymer having a formula of $HO(C_2H_4O)_n(C_3H_6O)_m(C_2H_4O)_nH$ with n having a value of about 80, with m having a value of about 27, and the poloxamer has an average molecular weight of from about 7680 to about 9510 g/mol.

22. The method of any one of claims 1-21, wherein the poloxamer is poloxamer 188.

23. The method of any one of claims 1-22, wherein the cell is a mammalian cell.

24. The method of claim 23, wherein the cell is a Chinese Hamster Ovary (CHO) cell.

25. The method of any one of claims 1-22, wherein the cell is an insect cell.

26. The method of any one of claims 1-25, wherein the cell produces a polypeptide.

27. A cell culture medium comprising a poloxamer prepared by the method of any one of claims 1-26.

28. The cell culture medium of claim 27, wherein the cell medium comprises the heat-treated poloxamer at about 0.1 g/L to about 10 g/L.

29. The cell culture medium of claim 28, wherein the cell medium comprises the heat-treated poloxamer at about 0.1 g/L to about 3 g/L.

30. The cell culture medium of claim 28, wherein the cell medium comprises the heat-treated poloxamer at about 3 g/L to about 10 g/L.

31. A method of producing a polypeptide in a cell culture, comprising the step of culturing a cell that produces the polypeptide in a cell culture medium under conditions suitable for production of the polypeptide, wherein the cell culture medium comprises a poloxamer prepared by the method of any one of claims 1-26.

32. The method of claim 31, wherein the cell is a mammalian cell.

33. The method of claim 31, wherein the cell is a Chinese Hamster Ovary (CHO) cell.

34. The method of claim 31, wherein the cell is an insect cell.

35. The method of any one of claims 31-34, wherein the cell medium comprises the heat-treated poloxamer at about 0.1 g/L to about 10 g/L.

36. The method of claim 35, wherein the cell medium comprises the heat-treated poloxamer at about 0.1 g/L to about 3 g/L.

37. The method of claim 35, wherein the cell medium comprises the heat-treated poloxamer at about 3 g/L to about 10 g/L.

38. The method of any one of claims 31-37, wherein the polypeptide is an antibody or antigen-binding fragment thereof.